# BIOSYNTHESIS OF STAPHYLOCOCCAL PHAGE ON CELL FREE BACTERIAL MEDIA

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Following is the translation of an article by P. A. Bulanov, Belorusskiy State University imeni V. I. Lenina, published in the Russian-language periodical Doklady Akademii Nauk BSSR (Doklady of the Belorussian Academy of Sciences), 1964, Vol VIII, No 9, pages 607--608. It was submitted on 20 Jun 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.

Up until now it was considered solidly established that viruses and phages as obligate parasites may multiply only in living cells which are susceptible to the given virus or phage. However, ~~as it turned out, this stable~~ assertion is already wavering at the ~~present time~~. Thus, ~~B. S. Diskina, A. V. Mikheyeva, Yu. Z. Gendon (2)~~ in ~~December~~ 1961 at the ~~XIVth~~ Scientific ~~Session of the Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, reported on~~ the possibility of the synthesis of the poliomyelitis virus from viral RNA in the system of destroyed cells of a monkey kidney with the addition of certain amino acids, nitrogenous bases and enzymes. In 1962 ~~the American virologists Wildman and Joung~~ Tak Kim (3, 5) ~~and also Conrat~~ (4) and co-authors ~~almost simultaneously published~~ reports on the multiplication of the tobacco mosaic virus in a system which did not contain living cells. Various nucleotides (adenosine-, guanosine-, cytidine- and uridene-triphosphates) were entered into this system. The addition to such a medium of the purified sap from plants which were afflicted with the tobacco mosaic virus causes the rapid multiplication of new viral particles.

In this way native and foreign scientists have shown the principle ~~possibility~~ of the biosynthesis of viruses on media which do not contain living cells, ~~which is a major achievement of contemporary~~ virology.

In respect to the viruses of bacteria (phages), analogous investigations have been conducted by us in the Microbiology Laboratory of the Institute of Experimental Botany and Microbiology, AN BSSR in 1963, and are being conducted at the present time in the Department of Microbiology of the Belgosuniversitet imeni V. I. Lenina. These investigations were preceded by our first work (1), accomplished already in 1958, on the cultivation of virus like formations of malignant tumors on cell-free nutrient medium, consisting of lysate of living cells of Sarcina. The lysing of Sarcina was accomplished with the help of lysozyme.

The tests on the biosynthesis of staphylococcal phage ~~we also~~ conducted on cell-free bacterial lysates.

Autonomous multiplication (reproduction) of phages may apparently be accepted only in specific microbial lysates, containing all the substances of the living microbial cells, including an energy producing system.

Based on the data of Khankok (1958), the cytoplasm of bacteria contains in a free state all the amino acids which go into its composition. Proline, glutamic acid, aspartic acid, isoleucine, methionine, and others were found in cultures of Staphylococcus aureus and other microorganisms in the stage of logarithmic growth. Stemming from this, it can be proposed that the lysate of Staphylococcus aureus contains free cytoplasmic amino acids which have been set free following dissolving by lysozyme of the wall of the bacterial cells and which pass into solution. Passing simultaneously into the solution are proteins, nucleic acids, fats, carbohydrates, enzymes, vitamins, carotinoid pigments, and other substances which are included in the composition of the living bacterial cells. However, in all probability the main components for the biosynthesis of phages are the low molecular compounds -- the amino acids which are found in microbial lysates. Besides this it is still necessary to have free energy, which the phages may obtain with the addition to the lysate of adenosinetriphosphoric acid (ATP), which is an unique energy accumulator. Under the influence of the enzyme -- adenosine triphosphatase, detected by B. F. Poglazovyy and A. S. Tikhonenko in the offshoots of phage particles, a break occurs in the bond between the phosphoric acids of ATP, and the energy included in it is set free and may be utilized by the phages for the development of their vital functions.

Method for the preparation of the cell-free bacterial medium. For the preparation of the given medium we used a culture of Staphylococcus aureus which is nonlysogenic and very sensitive to lysozyme (strain No 6), and which was isolated by us from the air.

From a 24-hour culture of this strain, incubated on glucose-liver agar, a suspension was prepared in an 0.5 percent solution of sodium chloride (pH 7.0). The concentration of the suspension was reduced to 2 billion microbial cells in 1 ml. To the staphylococcal suspension which was prepared in this manner we added an 0.1 percent solution of dry lysozyme in an amount of 0.2 ml per 100 ml of suspension. The complete lysis of the staphylococcal cells set in after one hour at 20--22°, after which a sterile filtration of the lysate was carried out through the asbestos plates of a Seitz filter. Subsequently it was poured into test tubes in the amount of 5 ml each and then we added 0.3 ml of a 1-percent solution of sodium salt of adenosine triphosphate to each test tube. The medium is prepared ex tempore.

Staphylococcal bacteriophage (obtained from the Tbilis Scientific-Research Institute of Vaccines and Sera, series No 5) in an amount of 0.2 ml was inoculated in the freshly prepared cell-free nutrient medium. For a control the same amount of phage was introduced into test tubes with 5 ml of meat-peptone broth. Cultivation was carried out in an incubator at 37°. The reseedings on fresh nutrient media were made every 48 hours in a volume of 0.2 ml of sown material. All told 20 passages were made.

The lytic activity of the phage cultures was checked on 5--6 hour broth cultures of an indicator strain of Staphylococcus aureus. The complete lysis of staphylococci was observed in 5--8 hours.

As our investigations showed, the phage cultures did not lose their lytic activity in all 20 passages on the cell-free bacterial medium, while in the control on meat-peptone broth the phage lost its activity already on the fourth passage.

Thus, the results of the tests testify to the feasibility of cultivating bacteriophages on specific cell-free substrates which do not contain living microbial cells.

It is fully understood that these data are only the first attempt at the creation of cell-free bacterial media for the biosynthesis of phages. In this direction further investigations are necessary for improving the method of preparing such media and the synthesis of other species of phages.

## Literature

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